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Efficacy of FDA-approved Hemostatic Drugs to Improve Survival and Reduce Bleeding in Rat Models of Uncontrolled Hemorrhage

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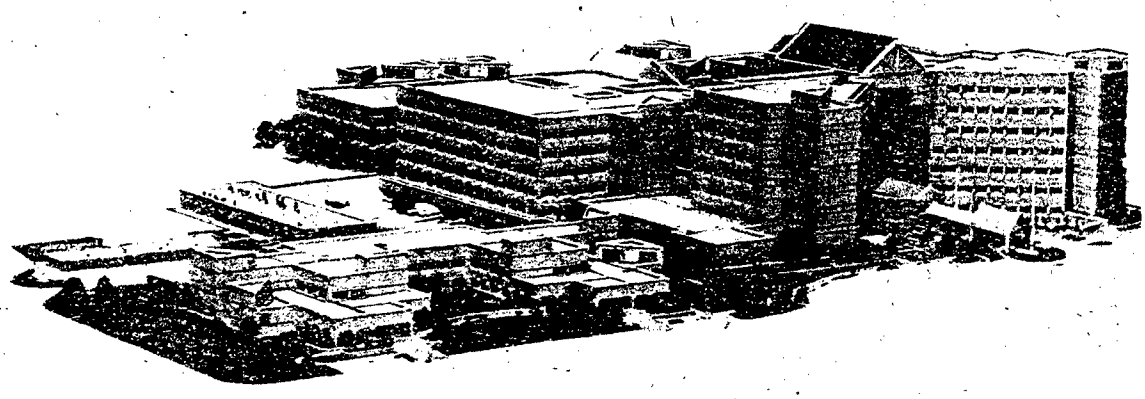

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14. ABSTRACT

Several FDA-approved intravenous drugs are used to reduce surgical bleeding. This series of studies tested whether these drugs (aprotinin, desmopressin, tranexamic acid, e-aminocaproic acid) could reduce bleeding due to traumatic injuries in two models of uncontrolled hemorrhage in rats. In the first phase of each study, a lethal liver injury was produced by excising a section of the median lobe (approximately 0.8% of body weight) and an infusion of either vehicle or the test substance was immediately begun. This model included aggressive fluid resuscitation and a severe dilutional coagulopathy. Blood loss, survival time and mortality rate were measured. Three studies were performed, testing each of the drugs singly and in combination. None of the drugs significantly reduced either bleeding time or blood loss in the tail bleeding model, nor were blood loss, survival time or mortality rate altered in the liver injury model. Taken together, these results suggest that these FDA-approved drugs, when used either singly or in combination, are not efficacious in these models of traumatic uncontrolled hemorrhage.

15. SUBJECT TERMS
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Efficacy of FDA-approved Hemostatic Drugs to Improve Survival and Reduce Bleeding in Rat Models of Uncontrolled Hemorrhage

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ABSTRACT

Several FDA-approved intravenous drugs are used to reduce surgical bleeding. This series of studies tested whether these drugs (aprotinin, desmopressin, tranexamic acid, ε-aminocaproic acid) could reduce bleeding due to traumatic injuries in two models of uncontrolled hemorrhage in rats. In the first phase of each study, a nonlethal tail bleeding model was used that incorporated limited fluid resuscitation (lactate Ringer's solution) and a mild dilutional coagulopathy. Four doses of vehicle or the test substance were administered successively with bleeding time and blood loss measured after each dose. In the second phase of each study, a lethal liver injury was produced by excising a section of the median lobe (approximately 0.8% of body weight) and an infusion of either vehicle or the test substance was immediately begun. This model included aggressive fluid resuscitation and a severe dilutional coagulopathy. Blood loss, survival time and mortality rate were measured. Three studies were performed, testing each of the drugs singly and in combination. None of the drugs significantly reduced either bleeding time or blood loss in the tail bleeding model, nor were blood loss, survival time or mortality rate altered in the liver injury model. Taken together, these results suggest that these FDA-approved drugs, when used either singly or in combination, are not efficacious in these models of traumatic uncontrolled hemorrhage.

Keywords: Trauma, emergency treatment, hemorrhage, intravenous hemostasis, liver injury, bleeding time

INTRODUCTION

The concept of using intravenous drugs to enhance or augment the body's innate clotting mechanisms during situations in which blood loss is expected is not new. Indeed, drugs have been used in the treatment of bleeding complications for over 30 years [1]. For example, the drugs aprotinin, desmopressin (DDAVP), epsilon-amino caproic acid (EACA), and tranexamic acid (TXA) have been used to reduce bleeding complications and blood loss in a variety of clinical situations including cardiac surgery, hepatic surgery, orthopedic surgery, and in patients with bleeding disorders [1-3]. Three of these drugs (aprotinin, EACA and TXA) are FDA-approved for use in perioperative hemostasis, while the fourth (desmopressin) has an approved indication for use in bleeding in patients with hemophilia or von Willebrand's Disease. Although many case reports documenting successful use of these drugs appear in the literature, it should be noted that the few randomized clinical trials investigating surgical use of these drugs have produced contradictory results [3-5].

Hemorrhage is a leading cause of death from traumatic injury [6], especially on the battlefield [7]. Many of these deaths are from noncompressible hemorrhage (i.e., that which is not accessible for manual pressure), for which there is currently no efficacious pre-surgical treatment. Finding an intravenous treatment that could assist endogenous clotting mechanisms is therefore a major mission for military researchers. The possibility that hemostatic agents might be useful in reducing blood loss following traumatic injury has received new impetus from the recent use of rFVIIa in both animal models of traumatic injury [8-14] and in human patients who have suffered traumatic injury [15-17]. Although rFVIIa holds promise for such indications, it is also very expensive and is not yet approved for a trauma indication. Because of this, the possibility that the much cheaper drugs aprotinin, DDAVP, EACA and TXA might be beneficial

in reducing traumatic bleeding was investigated using two models of uncontrolled hemorrhage in rats. This drug screening program was performed as a series of three studies, which investigated the efficacy of each of these drugs, used singly or in combination, to reduce blood loss in traumatic hemorrhage.

MATERIALS AND METHODS

General

A series of three studies was performed, each incorporating two separate experiments. In all experiments, animals were assigned randomly to treatment and investigators were blinded to treatment. The first experiment within each study incorporated a tail bleeding model with limited fluid resuscitation. This nonlethal model was considered the less hemostatically challenging of the two experiments. Because repeated bleeding time (BT) measurements were possible in this model, a dose escalation approach was possible, with the primary outcome measure being BT. The second experiment in each study employed a model of severe liver injury and aggressive fluid resuscitation. This was the more hemostatically challenging of the two and was selected to incorporate a rapidly developing dilutional coagulopathy. The primary outcome measures for this experiment were blood loss and survival. The specific experimental treatments and procedures are described below.

Animals

All experiments and animal care procedures were approved by the Institutional Animal Care and Use Committee, and the animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health publication 86-23,

revised 1996). The animals were maintained in a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International.

Male Sprague-Dawley rats (total n=222) were individually housed in standard plastic cages with water and food available ad libitum. Rats weighed between 400 and 450 g at the time of experiments. A 12:12-hr light-dark cycle (light on at 0600) was used, and the room temperature was maintained at 22-24°C.

Instrumentation

Anesthesia was induced by placing the rat in a sealed clear plastic box ventilated with 5% isoflurane in 100% oxygen. After induction, rats were removed from the box and placed in dorsal recumbency on a water-perfused heating pad. Anesthesia was maintained with 1-3% isoflurane in 100% oxygen via a nose mask throughout all surgical and experimental procedures. A temperature probe (Physitemp Instruments, Inc., Clifton, NJ) was inserted approximately 5 cm past the anus for measurement of colonic temperature, which was maintained at 37.5 ± 0.5°C throughout experimentation. For tail bleeding experiments, an additional temperature probe was placed subcutaneously 5 cm from the base of the tail to measure tail temperature. Arterial and venous catheters (PE-50) were placed via a femoral cutdown. The arterial catheter was used to monitor arterial blood pressure and for the withdrawal of blood samples, while the venous catheter was used for administration of hemostatic agents and fluid infusion. Mean arterial blood pressure (MAP), systolic and diastolic blood pressures, and heart rate were recorded at 10-second intervals throughout the study period using a continuous data collection system (Micro-Med, Louisville, KY).

Experimental Procedures

Tail Bleeding Experiment. Following placement of a temperature probe in the tail subcutis, the tail was laid on a custom-designed platform in which the tip of the tail was suspended. Tail temperature was maintained at 37.5 ± 0.5°C through the intermittent use of a radiant heat source in order to minimize variability in tail blood flow [18]. Figure 1 depicts the experimental timeline. An initial infusion of drug vehicle (lactated Ringer's solution, LR; 8.25 ml/kg body weight) was made over a 2 min period. Following a 3 minute circulation period, the distal 8 mm of the tail was removed by sharp excision using a scalpel blade. Blood was allowed to flow freely and to drip onto pre-weighed gauze; care was taken that the bleeding tip was not disturbed or touched in any way. The time to cessation of bleeding (bleeding time; BT) and the total amount of blood lost was measured and recorded. The first dose of the treatment (hemostatic agent or vehicle solution) was infused 15 minutes after the initial BT, again over a 2 minute period. Following a further 3 minute equilibration period, the distal 3 mm of tail was removed and BT and blood loss again measured. This sequence of events (drug or vehicle infusion followed by BT) was performed a total of four times, allowing the infusion of vehicle control followed by three doses of drug, with a 20 minute interval between each drug infusion. All doses reported are cumulative doses, taking into account the half-life of each drug. Arterial blood samples were collected prior to treatment administration and at 15 minutes after the start of the last BT measurement. At the end of the experiment, the animal was euthanized with sodium pentobarbital (150 mg/kg, i.v.). Tissue samples (brain, heart, lung, liver, kidney, and skeletal muscle) were collected and placed into formalin for histologic evaluation for evidence of intravascular coagulation. In pilot experiments using heparin and protamine, BT was found to be significantly related to coagulation function as measured by ACT (Figure 2).

Figure 1. Timeline for tail bleeding experiments.

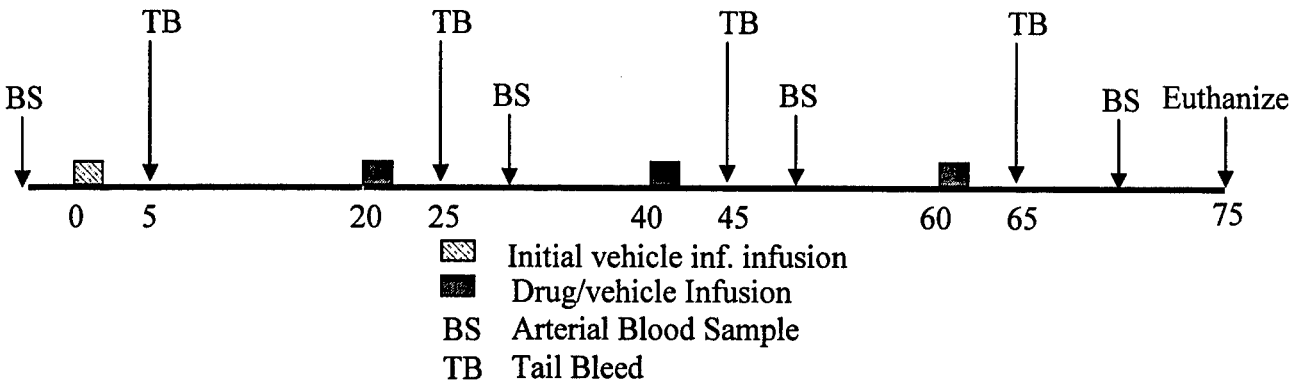
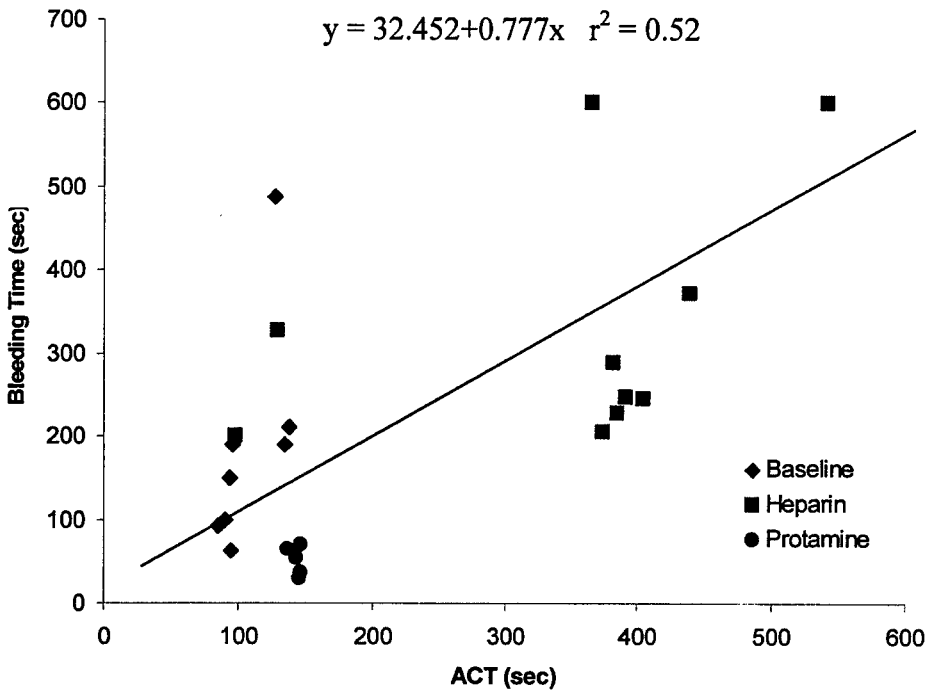


Figure 2. Correlation between ACT and tail BT (n=8).



Liver Injury Experiment. The liver injury model used was modified from that of Matsuoka et al. [19] and has been used previously in this laboratory [20, 21]. Briefly, a midline laparotomy was performed. With the use of a small plastic ruler, the capsule of the median lobe

of the liver was scored in three spots (lateral, medial, and on the midline), forming an arch 1.3 cm from the suprahepatic vena cava, with a handheld cautery. The abdominal cavity was wiped dry and a 5-minute stabilization period was begun. At the end of this period, the portion of the median lobe distal to the arch was sharply excised with scissors. The rapidly bleeding liver was not manipulated in any way, and the abdomen was rapidly closed using surgical staples.

Immediately upon completion of the liver injury, resuscitation with warm (40°C) LR (3.3 ml/min/kg) that contained the experimental treatment (total dose dissolved in 8.25 ml) was administered intravenously over 5 minutes. Following infusion of drug, infusion of LR (3.3 ml/min/kg) was continued until the pre-injury MAP was reached; infusion was intermittently continued throughout the study period to maintain MAP at this baseline level. After the liver injury, the animal was monitored for 60 minutes or until death, whichever came first. Arterial blood samples were collected immediately prior to liver injury and then at 5 minutes after liver injury. After 60 minutes, surviving animals were euthanized with sodium pentobarbital (150 mg/kg, i.v.).

At the end of the study period, the abdomen was reopened and intraperitoneal clots and fluid were removed with pre-weighed gauze sponges. The total blood loss for each animal was calculated as the difference between blood-soaked sponges minus the weight of the pre-weighed sponges. Total resuscitation fluid utilized and time to death were also recorded. The weight of the excised median lobe divided by the preinjury total body weight of the rat was used as a measure of the reproducibility of the injury [20, 21]. Tissue samples (brain, heart, lung, liver, kidney, and skeletal muscle) were collected and placed into formalin for histologic evaluation for evidence of intravascular coagulation.

Experimental Studies

Study 1 – Aprotinin and DDAVP. For the tail bleeding model (n=10/group), cumulative doses of aprotinin (Bayer Corporation, West Haven, CT) used were 0, 40,000, 80,000 and 120,000 KIU/kg. For DDAVP (Ferring Pharmaceuticals, Suffern, NY), cumulative doses were 0, 0.3, 1.5, and 3.0 μg/kg. Similar doses have been found to reduce drug-induced prolongation of bleeding time in rats [22-24] . For the liver injury model (n=15/group), doses of 120,000 KIU/kg and 3.0 μg/kg were used for aprotinin- and DDAVP-treated rats, respectively.

Study 2 – EACA and TXA. Both EACA and TXA were obtained in powder form from Sigma Chemicals (St. Louis, MO) and solubilized in LR. For the tail bleeding model (n=15/group), cumulative doses of EACA used were 0, 150, 300, and 450 mg/kg, while doses of 0, 100, 200 and 300 mg/kg of TXA were used. Again, similar doses have demonstrated antifibrinolytic activity in rats [23, 25, 26]. In the liver injury model (n=15/group), doses used were 450 mg/kg and 300 mg/kg of EACA and TXA, respectively.

Study 3 – Combinations. Because the various agents tested in studies 1 and 2 have different mechanisms of action (e.g, aprotinin inhibits fibrinolysis and may protect platelets, while DDAVP acts by release of vWF), it was conceivable that a combination of agents acting via different hemostatic mechanisms might be more efficacious than either agent alone. Two combinations were therefore tested in each model: aprotinin+DDAVP and aprotinin+EACA. Sample sizes were 10 and 9 rats per treatment group for the BT determination and liver injury model, respectively. Dose ranges used in the previous two studies were used for the tail bleeding phase in this study, with the drugs mixed together immediately before administration; likewise, the same maximal doses of each drug used previously were administered in the liver injury phase.

Laboratory procedures

Hematocrit (Hct), hemoglobin (Hb) and platelet (PLT) counts were performed as direct measurements using the ABX Pentra 120 hematology analyzer (ABX Diagnostics, Inc., Irvine, CA). ACT was performed using the Hemochron Junior (International Technidyne Corp., Edison, NJ) according to manufacturer's instructions (studies 2 and 3 only). Prothrombin time (PT) was determined using an automated coagulation analyzer (IL Futura, Lexington, MA, in studies 1 and 2; Dade Behring BCS system, Marburg, Germany, in study 3). Von Willebrand factor (vWF) concentrations were determined using the Asserachrom vWF micro enzyme immunoassay (Diagnostica Stago, Inc., Asnieres, France) for blood samples collected in the tail bleeding experiments only.

Pathology

Samples from brain, heart, lung, liver, kidney, and skeletal muscle were collected within 10 minutes of death and fixed in formalin. All samples were embedded in paraffin, sectioned, and stained using hematoxylin and eosin. Tissues were examined under light microscopy by a board-certified veterinary pathologist who was blinded to treatment group.

Statistical analysis

Data were analyzed using SAS, version 8.1 (SAS Institute Inc. 1999). Parameters at a single point were compared among groups using a one-way Analysis of Variance (ANOVA). Parameters with multiple measurements were compared using a hierarchical mixed model, in which the treatment, time, and treatment x time interaction were considered fixed factors, and the rat within treatment term was considered random. All data were tested for homogeneity of variance (PROC ANOVA with associated Levene's test) and normality of distribution (PROC Univariate Normal with associated Kolmogorov-Smirnov test). Data were transformed where

necessary to meet assumptions of ANOVA. Assuming there were no carry over effects and no time effects, BT, blood loss and ACT for the tail injury phase were analyzed using a mixed ANOVA with rat as a random block for the dose effect test within each group, and for the treatment effect test. A generalized Wilcoxon test was performed to compare survival times among groups. Multiple comparisons were adjusted by Tukey method. Analysis of covariance (ANCOVA) was used when including covariates. Data are presented as mean ± SEM and statistical significance was set at a value of $P<0.05$.

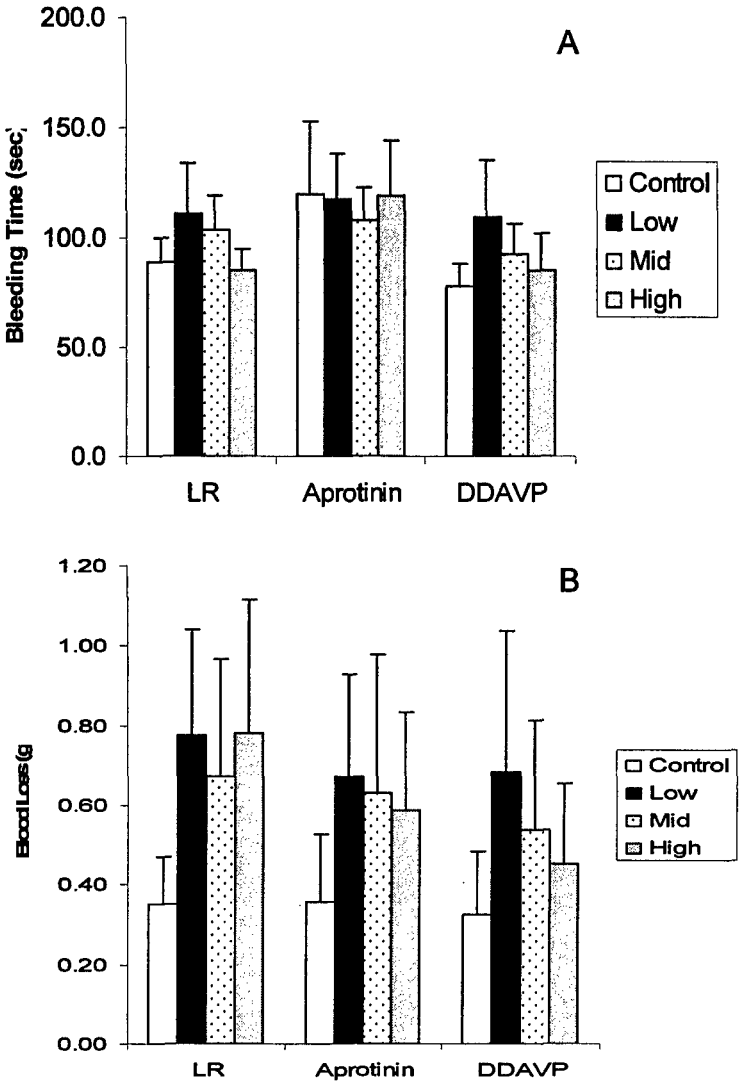
RESULTS

Study 1 – Aprotinin and DDAVP. For the tail bleeding experiment, there were no differences between the three groups at baseline in body weight, Hct, Hb, PLT, vWF, MAP, colonic temperature or tail temperature (all p values > 0.05; Table 1). Furthermore, there was no treatment effect evident in any of these variables at the end of the experiment. Within each group, there was no dose effect on BT (Figure 3). There were also no significant differences in BT when either aprotinin or DDAVP was compared with the vehicle control group at any dose level. As with BT, there were no differences in blood loss when either aprotinin (0.56 ± 0.25 ml) or DDAVP (0.50 ± 0.25 ml) was compared with the vehicle control group (0.64 ± 0.26 ml) at any dose level (Figure 3). No treatment effect on vWF was observed. PT was not different among treatment groups. Across treatment groups, PT was not prolonged significantly during the experimental period.

Table 1. Baseline values before BT determination and drug treatment in each group. ND, not detectable.

Parameter	**LR**	**Aprotinin**	**DDAVP**
Body weight (g)	439 ± 10	446 ± 10	435 ± 6
Hct (%)	36.8 ± 1.1	37.5 ± 1.1	37.7 ± 1.1
Blood [Hb] (g/dl)	12.8 ± 0.4	13.2 ± 0.4	13.1 ± 0.4
PLT (10^3/mm^3)	517 ± 18	551 ± 14	559 ± 18
PT (sec)	14.9 ± 0.7	15.4 ± 0.8	13.2 ± 0.7
TAT (μg/l)	ND	ND	ND
vWF (%)	39.6 ± 3.9	45.4 ± 4.6	34.8 ± 3.6
MAP (mm Hg)	95 ± 2	90 ± 1	89 ± 2
Colonic temperature (°C)	37.5 ± 0.1	37.6 ± 0.1	37.6 ± 0.1
Tail temperature (°C)	37.2 ± 0.1	37.3 ± 0.1	37.3 ± 0.1

Figure 3. BT (panel A) and blood loss (panel B) following each dose (low, mid, and high) of LR, aprotinin or DDAVP.



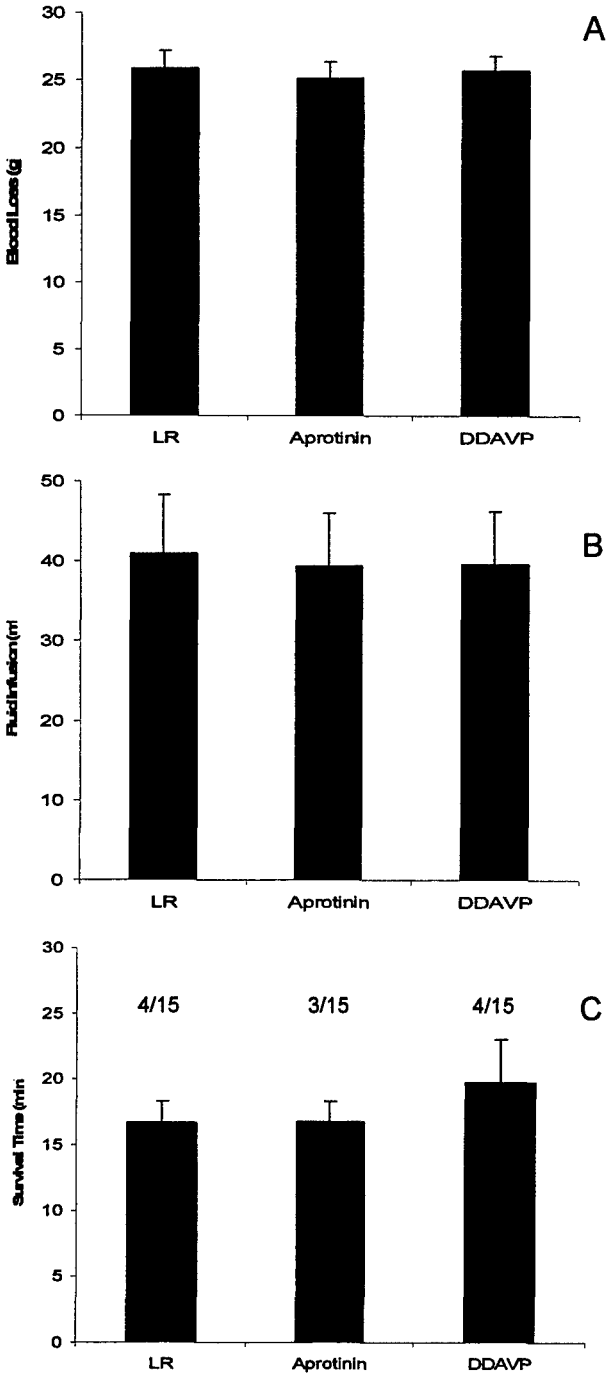
In the liver hemorrhage experiment, there were no significant differences in any of the baseline body weight, cardiovascular, temperature or hematological variables (Hct, Hb, PLT) among groups (Table 2). Following liver injury and resuscitation, there was no effect of treatment group on any parameter. Averaged across groups, Hct, Hb, and PLT decreased 46.9±1.6, 48.1±1.7, and 31.5±1.4%, respectively ($p<.01$), and PT increased 16.6 ± 0.1% ($p<.01$) by 5 minutes post-injury. The extent of liver injury produced did not differ between groups

(Table 2). This resulted in 1-hour survival rates of 26, 20 and 26% for vehicle, aprotinin and DDAVP groups, respectively; these rates did not differ (p=0.99). Finally, there was no treatment effect on blood loss, resuscitation fluid volume or survival time (Figure 4).

Table 2. Baseline values before liver injury and drug treatment in each group. ND, not detectable.

Parameter	**LR**	**Aprotinin**	**DDAVP**
Body weight (g)	430 ± 5	426 ± 4	431 ± 4
Hct (%)	36.6 ± 1.0	36.5 ± 1.0	35.9 ± 1.0
Blood [Hb] (g/dl)	12.7 ± 0.4	12.7 ± 0.4	12.6 ± 0.3
PLT (10^3/mm^3)	490 ± 24	500 ± 15	506 ± 14
PT (sec)	17.9 ± 0.6	17.7 ± 0.6	18.6 ± 0.6
TAT (μg/l)	1.7 ± 1.1	0.5 ± 1.1	ND
vWF (%)	34.2 ± 1.5	23.1 ± 3.0	34.1 ± 1.5
MAP (mm Hg)	89 ± 1	90 ± 1	88 ± 2
Colonic temperature (°C)	37.5 ± 0.1	37.7 ± 0.1	37.7 ± 0.1
Liver excised (% body weight)	0.82 ± 0.03	0.81 ± 0.03	0.82 ± 0.03

Figure 4. Blood loss (Panel A), fluid infusion volume (Panel B), and survival time (Panel C) following severe liver injury. In Panel C, the number of animals dying in each group is presented above the accompanying survival time; mean survival times do not include those of animals euthanized at 60 minutes.

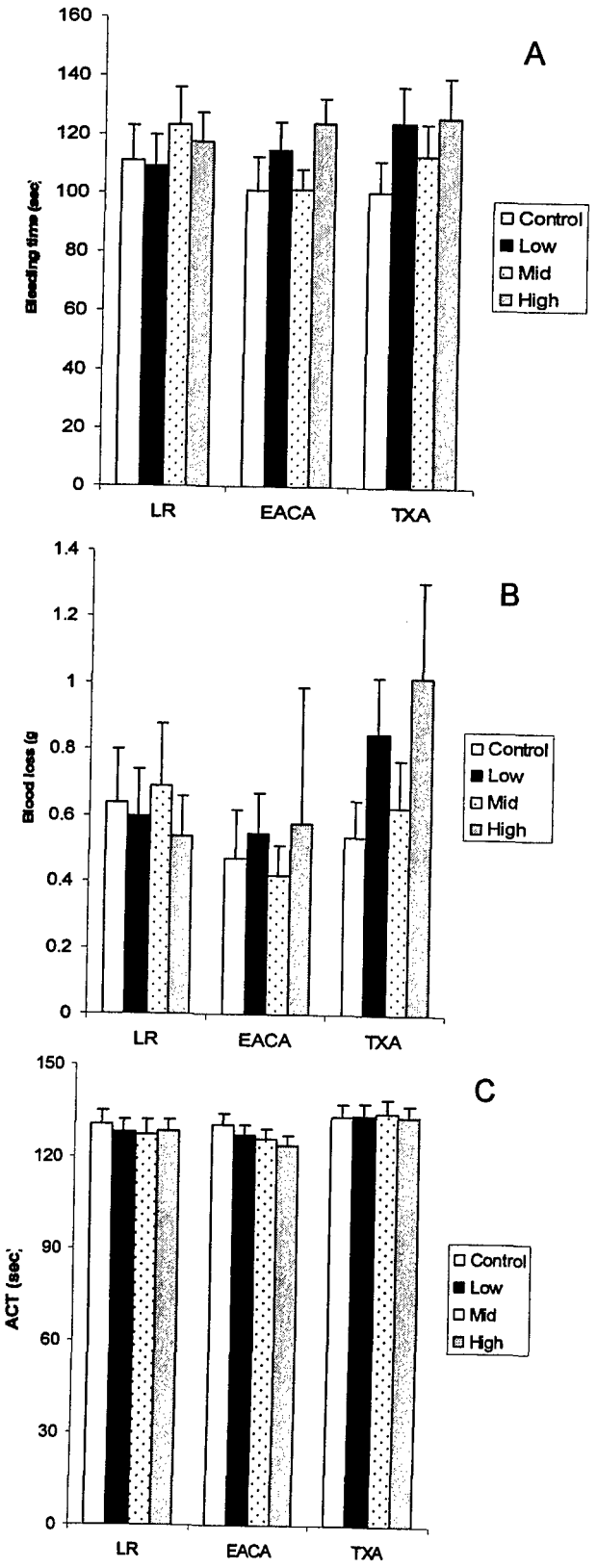


Study 2 – EACA and TXA. As before, baseline variables did not differ among treatment groups in either the tail bleeding (Table 3) or the liver hemorrhage (Table 4) experiments. There was no treatment effect evident in the majority of these variables at the end of the tail bleeding experiment, with the sole exception being a PLT value that was slightly increased in the TXA group relative to the vehicle control group ($p=0.02$). There were no dose-related differences in BT, blood loss or ACT within any treatment group (Figure 5). Furthermore, there were no treatment-related differences in any variable when either EACA or TXA was compared with the control group at any dose. Across treatments, PT increased 4.1 ± 0.1% ($p<.01$), while ACT did not change significantly ($p=.69$) during the experimental period.

Table 3. Baseline values before BT determination and drug treatment in each group.

Parameter	**LR**	**EACA**	**TXA**
Body weight (g)	435 ± 5	443 ± 6	434 ± 4
Hct (%)	38.3± 0.4	37.9 ± 0.4	37.9 ± 0.4
Blood [Hb] (g/dl)	13.4 ± 0.1	13.4 ± 0.1	13.3 ± 0.1
PLT (10^3/mm^3)	578 ± 18	555 ± 15	568 ± 16
PT (sec)	17.1 ± 0.2	16.9 ± 0.2	17.1 ± 0.2
ACT (sec)	128 ± 5	125 ± 5	128 ± 5
TAT (μg/l)	4.0 ± 1.6	2.4 ± 0.5	ND
MAP (mm Hg)	91 ± 2	92 ± 2	88 ± 1
Colonic temperature (°C)	37.5 ± 0.1	37.8 ± 0.1	37.5 ± 0.1
Tail temperature (°C)	36.5 ± 0.1	36.6 ± 0.1	36.4 ± 0.1

Figure 5. Bleeding time (panel A), blood loss (panel B) and ACT (panel C) following each dose (low, mid, and high) of LR, EACA or TXA.

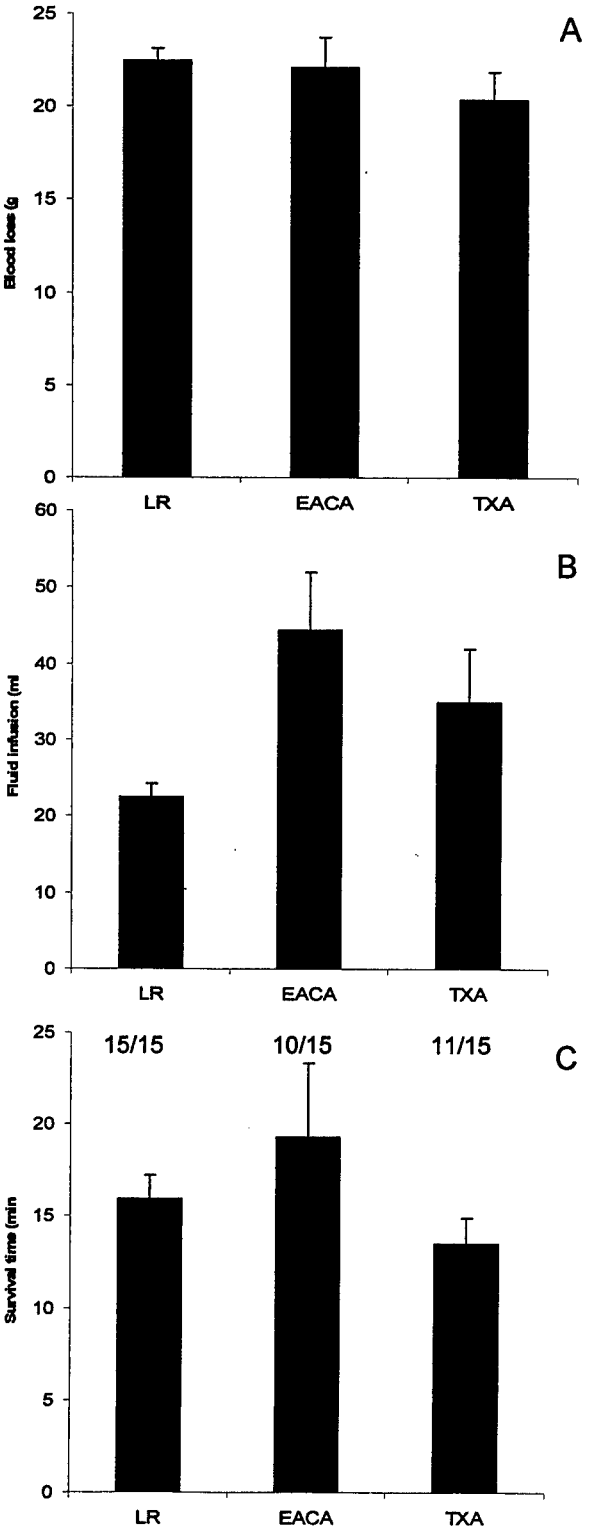


After liver injury, Hct, Hb, and PLT decreased over time across treatment groups with no differences among treatment groups observed. Again, the amount of liver excised did not differ among treatment groups. Survival rates following this severe injury were 0, 27 and 33% for vehicle, EACA and TXA groups, respectively, and did not differ. There was no treatment effect on blood loss, resuscitation fluid volume or survival time, although the difference in survival time between EACA- and vehicle-treated animals tended toward significance ($p=0.057$; Figure 6). There were no treatment effects on PT or ACT. Across treatments, PT was prolonged by $11.5 \pm 0.1\%$ ($p<.01$) and ACT increased $20.7 \pm 2.8\%$ ($p<.01$) during the observation period.

Table 4. Baseline values before liver injury and drug treatment in each group. ND, not detectable.

Parameter	**LR**	**EACA**	**TXA**
Body weight (g)	417 ± 2	423 ± 3	423 ± 3
Hct (%)	38.1 ± 0.3	37.4 ± 0.4	38.2 ± 0.5
Blood [Hb] (g/dl)	12.9 ± 0.4	13.0 ± 0.1	13.3 ± 0.2
PLT (10^3/mm^3)	487 ± 22	522 ± 18	487 ± 22
PT (sec)	17.9 ± 0.5	18.2 ± 0.4	18.5 ± 0.4
ACT (sec)	135 ± 2	135 ± 2	137 ± 2
TAT (µg/l)	2.8 ± 0.7	3.8 ± 1.4	1.8 ± 0.4
MAP (mm Hg)	91 ± 1	89 ± 1	87 ± 1
Colonic temperature (°C)	37.7 ± 0.1	37.7 ± 0.1	37.6 ± 0.1
Liver excised (% body weight)	0.91 ± 0.03	0.90 ± 0.03	0.89 ± 0.03

Figure 6. Blood loss (Panel A), fluid infusion volume (Panel B), and survival time (Panel C) following severe liver injury. In Panel C, the number of animals dying in each group is presented above the accompanying survival time; mean survival times do not include those of animals euthanized at 60 minutes.

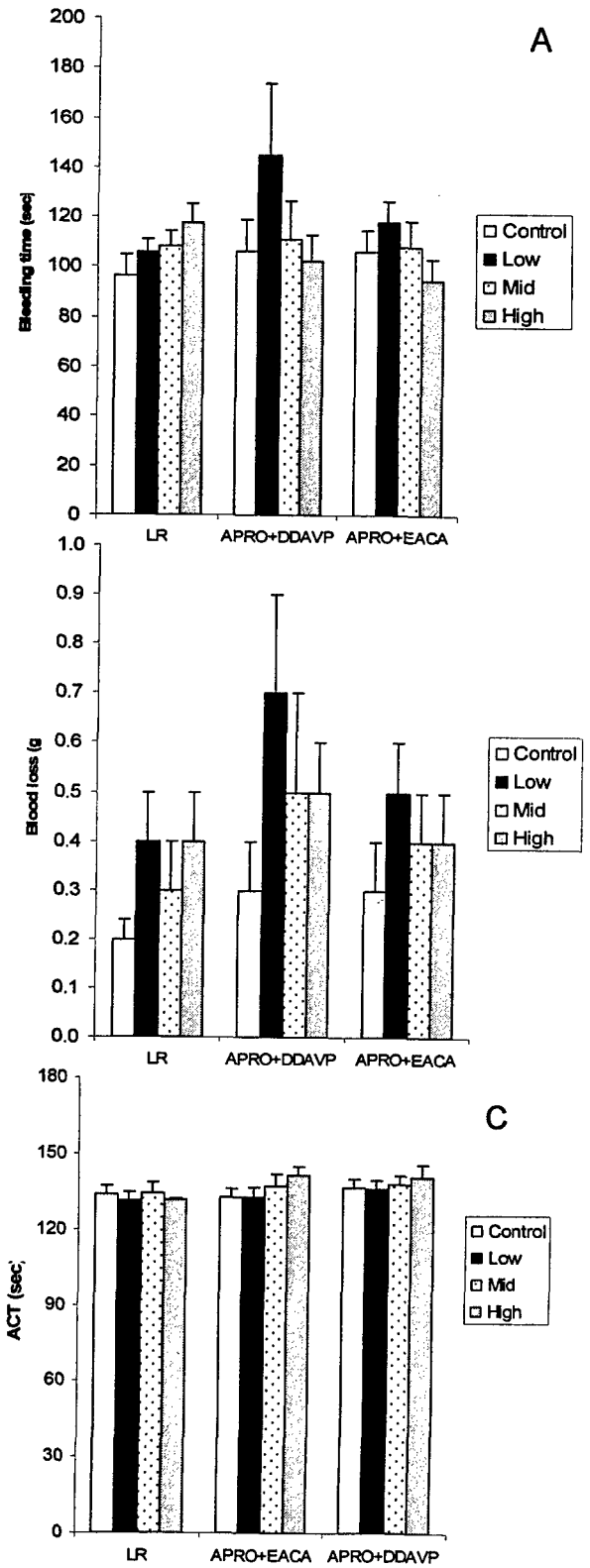


Study 3 – Combinations. No difference among treatment groups was detected in any baseline variable (Tables 5 and 6). In the tail bleed model, there were no differences among treatments in BT or blood loss at any dose examined. When adjusted for covariates, there were no dose-related differences in BT (Figure 7) within any treatment group. Despite this, blood loss from the initial BT determination in the vehicle control group (0.20 ± 0.04 ml) was lower than those at subsequent measurements (0.41 ± 0.10, 0.33 ± 0.07, and 0.44 ± 0.09 ml) within this group ($p<0.02$). Additionally, the lowest dose of aprotinin + EACA increased blood loss over the baseline level (0.51 ± 0.13 vs. 0.29 ± 0.08 ml; $p=0.03$); no other dose-related effects were observed within this group. Within the aprotinin + DDAVP group, there were no dose-related effects. BT, blood loss, and ACT did not differ between treatment groups at any dose level. There were no differences in PT or ACT among treatments at any time studied. When averaged across treatment groups, PT increased 3.3 ± 0.1% ($p=.01$) while ACT was unchanged during the experimental period.

Table 5. Baseline values before BT determination and drug treatment in each group.

Parameter	**LR**	**Aprotinin+DDAVP**	**Aprotinin+EACA**
Body weight (g)	421 ± 5	423 ± 5	421 ± 3
Hct (%)	39.3± 0.5	37.8 ± 0.5	38.3 ± 0.7
Blood [Hb] (g/dl)	13.8 ± 0.2	13.3 ± 0.2	13.5 ± 0.2
PLT ($10^3/mm^3$)	547 ± 19	501 ± 11	528 ± 23
PT (sec)	12.0 ± 0.2	11.9 ± 0.1	12.3 ± 0.2
ACT (sec)	136 ± 7	143 ± 4	138 ± 4
vWF (%)	35.3 ± 2.0	35.9 ± 1.9	31.1 ± 3.5
TAT (µg/l)	5.8 ± 3.2	3.2 ± 1.5	15.0 ± 7.0
MAP (mm Hg)	93 ± 3	96 ± 1	90 ± 2
Colonic temperature (°C)	37.8 ± 0.1	37.8 ± 0.1	37.8 ± 0.1
Tail temperature (°C)	36.4 ± 0.1	36.6 ± 0.2	36.5 ± 0.1

Figure 7. Bleeding time (panel A), blood loss (panel B) and ACT (panel C) following each dose (low, mid, and high) of LR, aprotinin+DDAVP, or aprotinin+EACA.

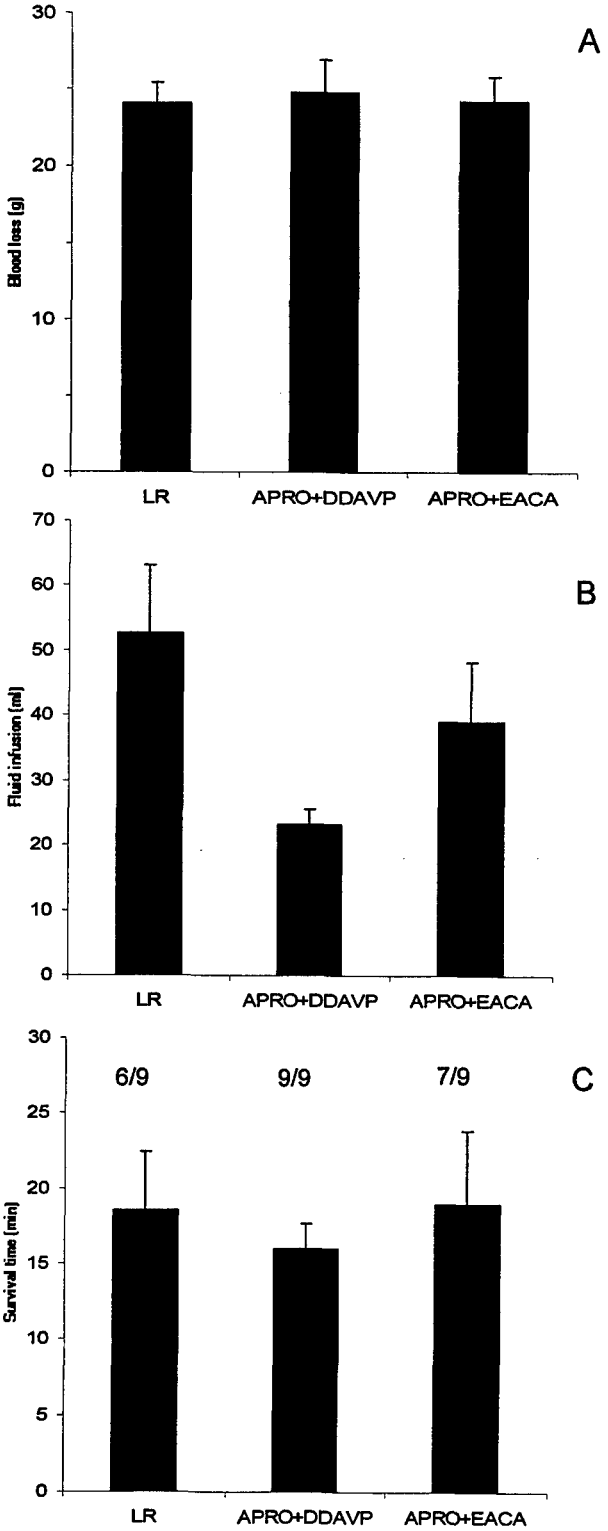


In rats subjected to liver injury, there were no differences in any baseline measurements among groups. Following liver injury, Hct, Hb, and PLT decreased over time without differences among treatment groups. The extent of liver injury produced did not differ among groups. This resulted in survival rates of 33, 0, and 22% for vehicle, aprotinin+DDAVP and aprotinin+EACA groups, respectively; these rates were not significantly different. Although survival time of the aprotinin+DDAVP group was numerically lower than that of the control group (Figure 8), this difference did not reach statistical significance ($p=0.06$). Neither blood loss (Figure 8) nor resuscitation volume used following liver injury was affected by drug treatment. There were no differences among treatment groups in either PT or ACT at any time. When averaged across treatment groups, PT was increased 25.2 ± 2.8% ($p<.01$) and ACT was prolonged by 58.6 ± 20.3% ($p<.01$) during the 60 minute experimental period.

Table 6. Baseline values before liver injury and drug treatment in each group.

Parameter	**LR**	**Aprotinin+DDAVP**	**Aprotinin+EACA**
Body weight (g)	427 ± 4	433 ± 4	423 ± 4
Hct (%)	36.5 ± 0.8	38.5 ± 0.7	37.5 ± 0.3
Blood [Hb] (g/dl)	13.0 ± 0.3	13.6 ± 0.3	13.3 ± 0.1
PLT (10^3/mm^3)	515 ± 23	511 ± 20	538 ± 21
PT (sec)	13.3 ± 0.5	12.1 ± 0.4	12.7 ± 0.2
ACT (sec)	134 ± 3	136 ± 5	127 ± 3
vWF (%)	29.4 ± 2.8	35.8 ± 1.8	35.6 ± 1.5
TAT (μg/l)	2.8 ± 0.9	4.0 ± 1.4	1.4 ± 0.2
MAP (mm Hg)	96 ± 2	90 ± 1	92 ± 2
Colonic temperature (°C)	37.7 ± 0.1	37.7 ± 0.1	37.7 ± 0.1
Liver excised (% body weight)	0.96 ± 0.04	0.96 ± 0.03	0.96 ± 0.03

Figure 8. Blood loss (Panel A), fluid infusion volume (Panel B), and survival time (Panel C) following severe liver injury. In Panel C, the number of animals dying in each group is presented above the accompanying survival time; mean survival times do not include those of animals euthanized at 60 minutes.



Pathological Analysis. No evidence of intravascular coagulation was noted during histopathological examination of collected tissues in any of the three studies. Perivascular edema was often observed in the lung and liver in all treatment groups (including vehicle control) and was probably due to infusion of large volumes of fluid.

DISCUSSION

The major finding of this series of studies is that none of the drugs or drug combinations tested were effective in decreasing BT or blood loss in two rat models of uncontrolled hemorrhage. Furthermore, there was no beneficial effect of any drug on increasing survival time or survivability in the more severe model of exsanguinating hemorrhage induced by liver injury.

In answering the question of whether these drugs would be beneficial in traumatic hemorrhage, we performed three replicates of each model of blood loss over a 28 month period. We were therefore able to compare data for control groups across these replicates to investigate the reproducibility of the models used. For BT determination, there were no differences between the replicates in either BT or blood loss. As with BT determination, there were no differences in data from vehicle control-treated rats between the three replicates of the severe liver injury model (Table 7). These data indicate that both models are highly reproducible. Furthermore, PT was slightly but significantly increased during the study period in 2 of 3 tail bleeding experiments (range 3.3% to 4.1%), while ACT was not altered. In the liver hemorrhage experiments, significant prolongations in both PT (range 11.5% to 25.2%) and ACT (range 20.7% to 58.6%) were observed in each study. All animals survived the tail bleeding procedure, while the majority of animals failed to survive the liver hemorrhage. We were therefore able to examine each experimental treatment in a nonlethal hemorrhage model incorporating limited fluid resuscitation and a mild dilutional coagulopathy, and also in a lethal hemorrhage model that

included aggressive fluid resuscitation and a severe dilutional coagulopathy. Previous work has demonstrated that the severe liver injury and resuscitation procedures used in this model are survivable with minimal blood loss if mechanisms (clamps or ligatures) are used immediately after injury [21], suggesting that a truly efficacious treatment administered at the time of injury would alter survival time.

TABLE 7. Endpoints in vehicle-control treated rats subjected to severe liver injury across the three studies. Mean survival times do not include those of animals euthanized at 60 minutes. There were no statistically significant differences among replicates in any endpoint.

Endpoint	**Study Replicate**		
	1	**2**	**3**
Liver excised (% of body weight	0.82 ± 0.04	0.90 ± 0.03	0.96 ± 0.04
Blood loss (ml)	25.9 ± 1.3	22.4 ± 0.7	22.1 ± 1.4
Survival time (min)	16.7 ± 1.6	15.9 ± 1.3	18.5 ± 3.9
Survival rate (%)	26%	0	33%

The drugs tested in this study act with a variety of mechanisms to enhance the endogenous coagulation system. DDAVP is a synthetic analogue of the neurohypophyseal hormone arginine vasopressin that increases factor VIII and vWF in plasma and enhances platelet adhesion to the vessel wall [3, 27]. In addition, DDAVP has a direct procoagulant action on platelets [28]. Aprotinin is a serine protease inhibitor. Although the mechanisms by which aprotinin decreases blood loss are not fully understood, data suggest that it inhibits fibrinolysis predominantly by inhibiting plasmin [3]. Additionally, aprotinin has been proposed to have a protective effect on platelets, although it does not directly affect platelet adhesion or aggregation [3]. Finally, EACA and TXA are lysine analogues that inhibit fibrinolysis by competitively blocking binding sites on plasminogen and hence its conversion into plasmin on the surface of fibrin [3, 4].

Two previous studies suggested the potential for aprotinin to decrease hemorrhage following trauma. In an early study, Araki and Lefer demonstrated that intravenous administration of aprotinin increased survival time in rats subjected to Noble-Collip drum trauma (a model for uncontrolled internal bleeding often accompanied by head injury). Although blood loss was not measured, rats which received aprotinin (20,000 or 40,000 KIU/kg) demonstrated elevated MAP relative to those that were not treated, suggesting a decrease in bleeding [29]. Subsequently, Thomae et al. [30] administered aprotinin to swine before and after performing a grade IV liver crush injury, without altering the volume of resuscitation fluid required to return blood pressure to baseline levels; all animals in both the control and aprotinin-treated groups survived for 4 hrs. When this crush injury was added to a controlled hemorrhage (to MAP of 20 mm Hg), aprotinin-treated animals demonstrated a decrease in mortality over that of the control group, although resuscitation volumes were not altered [30]. In this study, we were not able to demonstrate a beneficial effect of aprotinin in either a tail bleeding model or a model of severe hepatic hemorrhage. Drug dose may have been a factor but the dose used was similar to a dose that was used to reduce drug-induced bleeding in rats [22]. Neither TXA nor EACA reduced hemorrhage, despite the fact that the doses used have known antifibrinolytic activity in rats [25, 26]. Therefore, low dose is not likely to be the reason for lack of observed beneficial effects with these drugs.

DDAVP also failed to reduce bleeding in the present study. A dose of 1 ug/kg DDAVP reduced bleeding time in rats that were made coagulopathic by an overdose of an antithrombotic drug [24], presumably through a mechanism involving elevation of vWF. In our study, an elevation in plasma vWF was not detectable even after adjusting statistically for the potential dilutional effect of the volume of drug infused. This lack of elevation of vWF could account for

the lack of hemostatic efficacy observed. An elevation in vWF was expected because others had previously demonstrated such an effect with a similar dose in rats [31]. It should be noted, however, that an elevation in vWF does not appear to be absolutely required for a hemostatic effect [32]. However, we cannot rule out the possibility that our inability to demonstrate efficacy was because the DDAVP dose was not adequate.

Because hemostasis is a balance between the processes of both coagulation and fibrinolysis, we considered the possibility that hemorrhage control might be enhanced by influencing either procoagulant or fibrinolytic processes. Clearly, results from the present study do not support the contention that any of the investigated drugs would be useful in reducing exsanguinating blood loss in patients with normal coagulation function. First, there was no diminution of BT at any dose level for any of the administered drugs. Second, administration of these drugs either singly or in combination did not decrease blood loss or increase survival time following severe liver injury. Furthermore, resuscitation fluid volumes required to raise MAP to baseline levels were not significantly affected by drug treatment. Results suggest that inhibition of fibrinolysis does not have potential to improve hemostasis under the conditions of the models used. The potential enhancement of processes relating to clot formation attempted by enhancing platelet function or plasmatic procoagulant activity were also not effective.

CONCLUSIONS

The administration of the drugs aprotinin, DDAVP, EACA and TXA, either singly or in combination, did not alter BT or blood loss in two models of uncontrolled hemorrhage in rats without pre-existing coagulopathy. Furthermore, these drugs did not produce beneficial effects on either survival time or survival rate in a severe liver injury model relevant to lethal trauma. We therefore conclude from this series of experimental studies that these drugs were not useful

in enhancing the hemostasis in these highly consistent models of uncontrolled hemorrhage, and may not prove useful in pre-hospital treatment of exsanguinating hemorrhage.

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